Supporting Information

Heterogeneous Films of Ionotropic Hydrogels Fabricated From Delivery Templates of Patterned Paper

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Contents

Experimental

Materials: Sodium alginate, ι-carrageenan, the supplies for protein expression, and the inorganic salts used to cross-link the gels were purchased from Sigma-Aldrich (Milwaukee, Wisconsin) or VWR International (West Chester, Pennsylvania) unless otherwise noted. These materials were used without further purification. Solutions of sodium alginate (2% w/w), calcium chloride, calcium nitrate, ferric chloride, holmium nitrate pentahydrate, gadolinium chloride, copper nitrate, nickel nitrate, and barium chloride were made from 18 MΩ·cm deionized water. Whatman No. 1 chromatography paper was obtained from Sigma-Aldrich in square sheets measuring 20 × 20 cm. Sheets of transparency film were obtained from 3M (Item #CG3460) or OfficeMax (Itasca, Illinois, Item #OM96386).

Construction of the Templates: A Xerox Phaser color laser printer (Model 6250) printed patterns of toner onto chromatography paper to form the templates. We printed each pattern onto the same sheet of paper three times and heated the sheet with a heat gun at ~200 °C for 30 seconds to seal cracks and holes in the layer of toner. As an alternative to toner, we applied packaging tape (Staples® Clear View packaging tape) to the paper as a hydrophobic barrier. In designing templates for production of the heterogeneous films, we used Adobe Illustrator to draw patterns and imported these patterns to a laser cutter (Versa Laser cutter model VLS3.50). In the settings menu of the associated software package, we selected the type of material as "plastic (microsurface–deep engraving)" and entered the thickness of the material as 1.4 mm (a value much greater than the actual thickness of the paper) to ensure that the desired cuts penetrated the entire sheet. The laser settings were set to 0% raster, 0% vector marking, 0% vector marking (on a scale from −50% to 50%). These settings were sufficient for all of the sheets we patterned with

the laser cutter: chromatography paper with toner, chromatography paper with tape, and transparency film.

Thickness Measurements: The thicknesses of the hydrogel films were measured with a pair of digital calipers; the final values are reported as 90% confidence intervals based on the average of three trials.

Gel-in-Gel Structures Containing Different Cross-linking Ions (Dry Method): To create structures of Ca^{2+}–AA surrounded by Fe^{3+}–AA, we used the laser cutter to cut shaped holes through a piece of chromatography paper with toner printed on one side. We wet the sheet completely with a 1 M solution of $FeCl_3$ (on the side that did not contain toner), and wet a second piece of unpatterned chromatography paper (this sheet did not contain toner or holes) with a 2 M solution of $CaCl_2$. We dried the sheets with a stream of air from a heat gun (~35 °C), and placed the sheet with $FeCl_3$ on top of the sheet with $CaCl_2$ such that the toner was sandwiched between the two sheets to prevent aqueous solutions on the layers from mixing. The desired shape of the perimeter for the film was cut into a transparency film with the laser cutter, and this sheet was stacked on top of the layered paper template. The stack was held flat against a Petri dish (or any convenient sturdy, flat surface) with adhesive (either Scotch® tape or UHU-brand glue stick), and an aqueous solution of sodium alginate (2%, w/w) was poured onto the template. After 2 minutes, we washed the uncross-linked polymer away and used a spatula to remove the heterogeneous film from the template. If fibers of paper adhered to the bottom face of the gel, the film was immersed in water for several minutes until the fibers loosened such that they could be gently rubbed away from the gel by hand. To create the inverse pattern—structures of Fe^{3+}–AA surrounded by Ca^{2+}–AA—we carried out the same procedure except for wetting the first (top) sheet with 2 M $CaCl_2$ and the second (bottom) sheet with 1 M $FeCl_3$.

Gel-in-Gel Structures Containing Different Cross-linking Ions (Wet Method): To create a film in the shape of a star composed of a region of Ca^{2+}–CG and a region of Fe^{3+}–CG (see Figure 3), we used the laser cutter to cut a star shape into a sheet of transparency film and scissors to cut a long rectangle of chromatography paper backed with toner on one side. An unpatterned piece of chromatography paper (this sheet did not contain toner or holes) was backed with duct tape and pasted flat against a plastic Petri dish (or any convenient sturdy, flat surface). Paste was applied to the side of the patterned chromatography paper with toner, and this piece was glued on top of the unpatterned sheet. Finally, the transparency film was pasted on top of the stack. With a 100 μL micropipettor, we wet the bottom layer of paper with aqueous 2 M $FeCl_3$ and the top layer with 2 M $CaCl_2$ (Figure 3b). An aqueous solution of ι-carrageenan (2%, w/w) poured onto the template gelled into the desired film within 2 minutes, and we washed the excess (uncross-linked) polymer away with tap water. We peeled away the transparency film from the Petri dish and used a metal spatula to remove the heterogeneous film from the template.

Gels Containing Spatial Step-Gradients in the Concentration of Cross-linking Ions: To make a film of Fe^{3+}–AA with a gradient in cross-linking density, we used a pair of scissors to cut a piece of chromatography paper with toner printed on one side into several rectangles. Each square contained a different concentration of $FeCl_3$ solution: 0.2 M, 0.8 M, or 3.0 M. We formed a continuous template by placing the pieces next to each other in order of increasing ion concentration with a small amount of overlap (~2 mm) and poured 2% AA on top of the template. We washed the uncross-linked polymer away after 2 minutes and used a spatula to carefully remove the gel from the paper.

Biological Experiments: Stock solutions were made from deionized water unless otherwise indicated. Liquid LB medium: 5 g/L yeast extract, 10 g/L peptone from casein,

5 g/L NaCl. Isopropyl β-D-1-thiogalactopyranoside (IPTG): 48 mg/mL (0.2 M). X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside): 80 mg/mL (0.2 M) in *N*,*N*-Dimethylformamide (DMF). LB-agar plates were poured from an autoclaved solution of 20 g/L agar in LB medium to which 100 mg/L of sodium ampicillin was added after the solution had cooled below 55 °C.

E. coli Transformation and Culture: We transformed the plasmid pUC18 (Stratagene, La Jolla, California) into *Escherichia coli* BL21gold(DE3) (Stratagene) using the protocol supplied by the vendor. The bacteria were cultured in liquid LB and on LB-agar plates at 37 °C.

Controlled Growth of Bacteria in Hydrogel Films: We tested the growth of bacteria in homogeneous films of Ca^{2+}–AA, Ba^{2+}–AA, Ni^{2+}–AA, Cu^{2+}–AA, Al^{3+}–AA, Ho^{3+}–AA, and Gd^{3+}–AA. The polymer stock solution used to make the films contained AA (20 g/L), yeast extract (10 g/L), peptone from casein (20 g/L), and NaCl (10 g/L). Prior to formation of the films, 500 μL of 0.2 M IPTG and 500 μL of 0.2 M X-gal were added to a 35 mL aliquot of the solution of AA–LB and the mixture was shaken vigorously. Once the air bubbles in the solution had dissipated, 3 mL of a liquid culture of *E. coli* (O.D. = 1.0–1.5 at 600 nm for a pathlength of 10 mm) was added and the mixture was rocked gently so as not to introduce new bubbles of air. Circular films were made from this solution, placed in polystyrene culture trays, incubated for 6 hr at 37 °C, and photographed.

For screening the toxicity of homogeneous films of M^{n+}–AA, we formed the films using solutions that were 1 M in $CaCl_2$ and 1 M in $Ni(NO_3)_2$, $Cu(NO_3)_2$, $BaCl_2$, $Al(NO_3)_3$, $Ho(NO_3)_3$, and $GdCl_3$. As control experiments, we also formed films with 2 M $CaCl_2$ and 2 M $Ca(NO_3)_3$ to

demonstrate that the toxicity of the cross-linking solutions arose from the metal ions and not the counteranions.

We tested the growth of bacteria on heterogeneous gels of AA cross-linked with aluminum ions and calcium ions. To create a square structure of Al^{3+}–AA + Ca^{2+}–AA surrounded by a hexagon of Ca^{2+}–AA, we used the laser cutter to cut a square hole through a piece of chromatography paper with toner printed on one side and a hexagon-shaped hole into a sheet of transparency film. In sequence, we glued a sheet of unpatterned chromatography paper to a Petri dish, glued the patterned piece of chromatography paper on top of the first sheet, and glued the transparency film on top of the stack such that the template looked like that shown in Figure 5a. We wetted the bottom piece of paper with a solution of 1 M $Al(NO_3)_3$ + 1 M $CaCl_2$, and the top sheet with 2 M $CaCl_2$. We poured the AA–LB solution onto this new template, waited for 2 min., washed away the uncross-linked polymer, and used a spatula to peel the heterogeneous gel from the template. The film was incubated for 6 hr at 37 °C.

Figure S1. A schematic diagram of a typical delivery template used in the production of heterogeneous films of ionotropic hydrogels. The template shown matches that of Figure 1e. In the cross-sectional view, the scale of the z-dimension of the layers (i.e., the thickness of each layer) is exaggerated relative to the x- and y-dimensions.

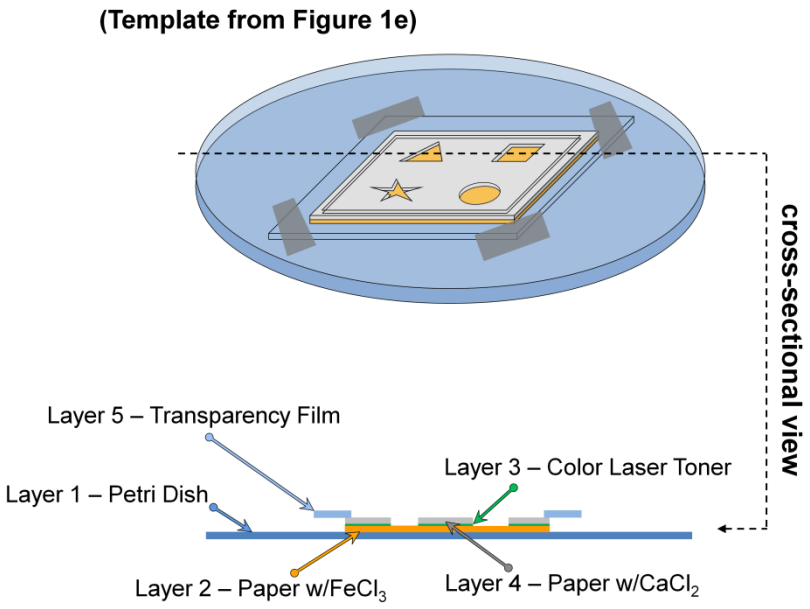

Figure S2. A schematic diagram showing the steps for the fabrication of gels with step-gradients in the concentration of cross-linking ions. A sheet of chromatography paper with a printed layer of toner (a) was cut into shaped pieces (b). We turned these pieces over and wet them with solutions of salts at different concentrations (c) and juxtaposed the pieces with ~2 mm of overlap. A 2% solution of sodium alginate applied to the template (d) gelled into a single continuous film (e).

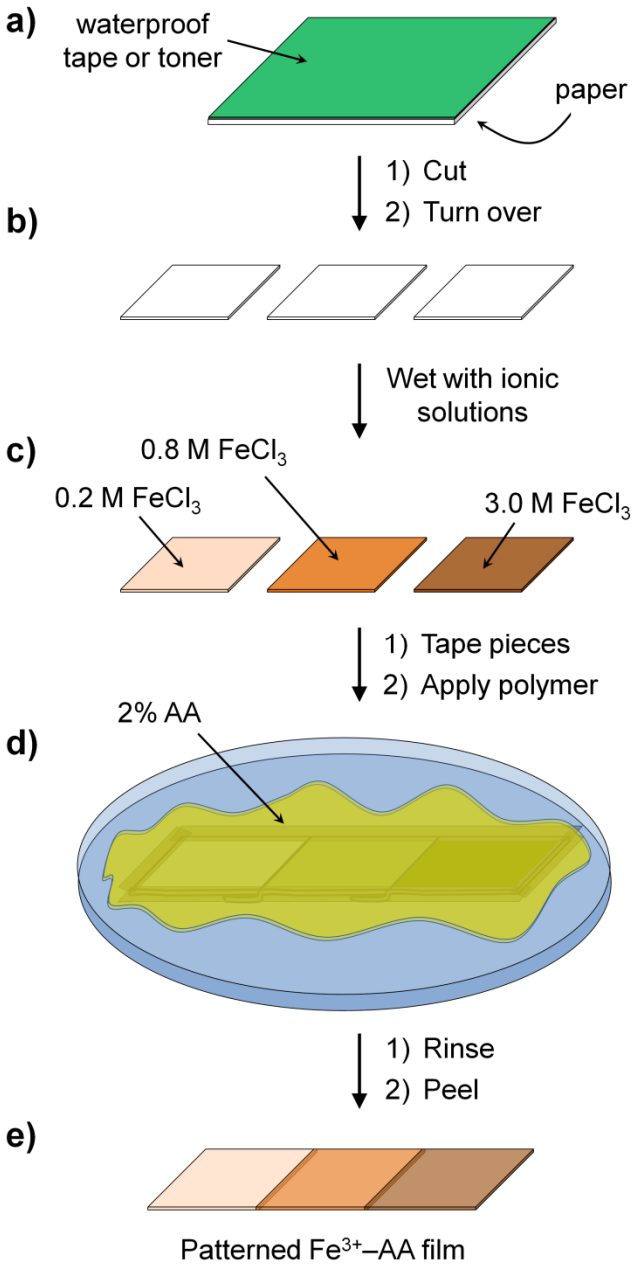

Figure S3. Manipulation of a patterned hydrogel film with a bar magnet. a) An arrow-shaped film with a central strip of Ho^{3+}–AA aligned with the long-axis of a bar magnet positioned beneath the container of the film. An anionic red dye, Allura Red AC, added to the solution of sodium alginate used to form the film improved the contrast of this image. b) Top view of the template used to produce the arrow-shaped magnetic film.